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APPLICATION No.

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PATENT

INVENTOR: *PATRICK T. PRENDERGAST*

TITLE: *FORMULATION TECHNIQUES FOR THE PREPARATION OF COLLOIDAL SUSPENSIONS FOR THERAPEUTIC ADMINISTRATION.*

OPEN TO PUBLIC INSPECTION UNDER SECTION 28 AND RULE 23

JNL. No. 1800 OF 14/11/96

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31/565

BACKGROUND

DHEA in its native state is non soluble in water, physiological saline or dextrose. Herein is described the preparation of therapeutic formulations which allow DHEA to be administered to a patient in a colloidal suspension. This allows, for the first time, a pharmaceutical formulation containing DHEA to be administered I.V. as a micronized particle colloidal suspension. This technical achievement was recorded following extensive research. Intravenously injected colloidal particle suspensions become localised in tissues of the reticuloendothelial and lymphoid system in mammals. The phagocytic tissue macrophages form a network - the reticuloendothelial system (RES) which is found in many organs. The lymphoid system is comprised of lymphocytes, epithelial and stromalcells and is arranged either into discreetly capsulated organs or accumulations of diffuse lymphoid tissue. Promonocytes in the bone marrow give rise to the blood monocytes and these represent a circulating pool which migrate into the various organs and tissue systems and become macrophages. The cells of this system include circulating blood monocytes and dispersed phagocytes in connective tissue such as Kuffer cells in the liver or fixed to the endothelial layer of the blood. The human

blood monocyte is a large cell (10 - 18 um diameter) relative to the lymphocyte. Lymphoid organs contain lymphocytes at various stages of development and are classified into either primary/central lymphoepithelial organs or secondary/peripheral lymphoid organs. The primary lymphoid organs are the major sites of lymphopoiesis. Here, lymphocytes differentiate from lymphoid stem cells, proliferate and mature into functional effector cells. In mammals, including man, T lymphocytes are produced in the thymus and B lymphocytes in the foetal liver and bone marrow. In avian species there is a specialised site of B cell generation, the bursa of Fabricius. In the primary lymphoid organs the lymphocytes acquire their repertoire of specific antigen receptors in order to cope with the antigenic challenges the individual receives during its life. They also learn to discriminate between self antigens, which are tolerated, and non-self antigens which, generally are not. Secondary lymphoid organs include lymph nodes, spleen and mucosal associated tissue including the tonsils and Peyer's patches of the small intestine. The secondary lymphoid tissue creates the environment in which lymphocytes can interact with each other and with antigens and disseminates the immune response once

generated. These functions are performed by phagocytic macrophages, antigen-presenting cells, and mature T and B lymphocytes in the secondary lymphoid organs.

Recent studies have clearly shown that the fatty acid content of red blood cells and white blood cells are abnormal in immune challenged patients. This abnormality is particularly clear when the results are analysed and expressed as the ratio between stearic and oleic acid concentration (saturation index). It has also been demonstrated that ethanol extracts of plasma from patients suffering immune challenge can cause considerable modification of various immune responses, especially lymphocyte viability and the T4/T8 ratio. This action is mimicked in-vitro by free unsaturated fatty acids such as linoleic acid.

The action of the unsaturated fatty acids is dose-dependent in causing a negative effect on lymphocyte viability. When Cortisol is added to this free unsaturated fatty acid it greatly enhances reduction of T4/T8 ratio.

Increases in polyunsaturated fatty acids can greatly inhibit Cortisol metabolism. This decreased

metabolism by lymphocytes can explain the elevation in serum Cortisol, decreased corticosteroid concentration and enhanced urinary free Cortisol in immune challenged patients. It has also been shown that these polyunsaturated fatty acids can inhibit aldosterone biosynthesis by depressing the binding of angiotensin to its adrenal receptor thus reducing DHEA synthesis.

After administering ACTH to healthy humans it was found that DHEA was conjugated to form sulphatidyls or phosphatidyls. One molecule of free DHEA binds one molecule of sulphuric or phosphoric acid, one molecule of glycerol and two molecules of free fatty acid, most frequently oleic or linoleic. It is assumed that DHEA circulates in the plasma mainly in this form as DHEA sulphatidyls, and this type of 4 conjugate of DHEA persists longer in plasma than free DHEA or DHEAS. Thus the level of free DHEA available in the plasma of a patient determines the amount of free unsaturated fatty acids that will be absorbed in DHEA sulphatidyl synthesis thus reducing the slide in saturation index that accompanies the progression to immunosuppression.

Therefore, if administered DHEA is to be available for sulphatidyl synthesis it must be prevented from

entering the inactive state of DHEAS. Since DHEAS levels are greater than control values in certain individuals who have been demonstrated to be prone to immunosuppression their free DHEA levels are reduced, their capacity to buffer the effects of viral infection with its resultant release of free unsaturated fatty acids is limited due to deficient resources of free DHEA to form sulphatidyls.

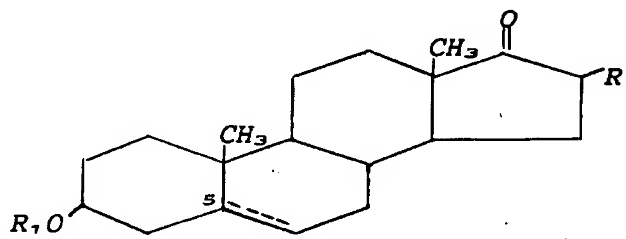
To achieve maximum anti-viral potency using DHEA it is necessary to avoid production of the DHEA sulphate form and to maximise the delivery to the cell of free DHEA or the more potent anti-viral DHEA sulphatidyls or phosphatidyls form.

PRESENT EMBODIMENT

DHEA in its native state is non soluble in water, physiological saline or dextrose. Herein is described the preparation of therapeutic formulations which allow DHEA to be administered to a patient in a colloidal suspension. This suspension is preferentially targeted to the lymphatic system with low residence time in the blood system. This allowed, for the first time, a pharmaceutical formulation containing DHEA to be administered I.V. as a micronized

particle colloidal suspension. This achievement was recorded following exhaustive research.

The compound having the general formula (I)



(I)

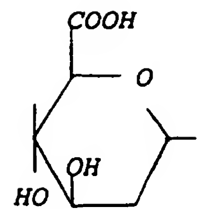
in which R is a hydrogen or bromine atom, and R_1 is a hydrogen atom, an SO_2OM group wherein M is a hydrogen or sodium atom, a sulphatide group

$$-SO_2O-CH_2.\underset{\displaystyle |\atop \displaystyle O.CO.R_2}{CH}.CH_2O.CH.R_3$$

a phosphatide group

$$-\overset{\displaystyle O\atop \displaystyle \|}{\underset{\displaystyle \|\atop \displaystyle O}{P}}-O.CH_2.\underset{\displaystyle |\atop \displaystyle O.CO.R_2}{CH}.CH_2.O.CO.R_3$$

wherein each of R_2 and R_3, which may be the same or different, is a straight or branched chain alkyl radical of 1 to 14 carbon atoms, or a glucuronide group



wherein the broken line represents an optional double bond, and the hydrogen atom at position 5 is present in the α- or β- configuration or the compound comprises a mixture of both configurations.

When R_1 is other than a hydrogen atom, the compounds are conjugated compounds.

Preferably in the compound of formula (I), R and R_1 are each hydrogen. An especially preferred compound is dehydroepiandrosterone wherein R and R_1 are each hydrogen and the double bond in present.

In a further embodiment of the invention, the compound is 16α-bromoepiandrosterone, wherein R is Br, R_1 is H and the double bond is present. In a still further embodiment of the invention, the compound is etiocholanolone wherein R and R_1 are each hydrogen and the double bond is absent.

Other preferred compounds are dehydroepiandrosterone sulphate, wherein R is H, R_1 is SO_2-OM and M is as hereinbefore defined and the double bond is present, and 5ß-androstan-3a-ol-17-one.

Alternatively, the compound is selected from dehydroepiandrosterone sulphatides, phosphatides or glucuronide wherein R is H, and R_1 is a sulphatide, phosphatide or glucuronide group as hereinabove defined, and the double bond is present. In particular when R_1 is not hydrogen the compounds are DHEA conjugates such as hexyl sulfate, dodecyl sulfate, octadecyl sulfate, octadecanolyglycol sulfate, O-dihexadecyclglycaro sulfate, hexadecane sulfonate, dioctadecanoylglycero phosphate, O-hlhexadecylglycero phosphate.

DHEA in its native state is non soluble in water, physiological saline or dextrose. Herein is described the preparation of therapeutic formulations which allow DHEA to be administered to a patient in a colloidal suspension. This allowed, for the first time, a pharmaceutical formulation containing DHEA to be administered I.V. as a micronized particle colloidal suspension. This achievement was recorded following

extensive research.

The formulation contains block copolymer of ethylene oxide and propylene oxide with 500ppm w/w Butylated hydroxytoluene {2,6-di-tert-butyl-4-methylphenol} (BHT) added as antioxidant (polyxamer 407 -"Synperonic" trade mark of ICI) in addition to DHEA. The DHEA is preferably in a micronized form. In addition the above formulation may also contain the pharmaceutical agent Metoclopramide to enhance the therapeutic benefit of the formulation.

Both ethylene oxide and propylene oxide may be used as polyadditive nonionic surfactants. The oxyethylene unit imparts hydrophilic properties: for a given hydrophobic chain R, the number of oxyethylene units added will determine the water solubility, which gives wide freedom in adjusting the properties according to the hydrophobic chain and the rate of polyalkoxylation. Block polymers are known as "pluronics" (CTFA: poloxamers) they are polycondensates of ethylene oxide and propylene oxide, of general formula:

$$HOCH_2-CH_2(CH_2-CH_2O)_a-(\underset{\displaystyle CH_3}{\underset{|}{CH}}-CH_2O)_b-(CH_2-CH_2O)_c-H$$

Propylene oxide brings some hydrophobic properties. In these compounds the fatty chain R is replaced by a propylene oxide polyadduct, the hydrophilic properties being given by the adjunction to each end of a number of oxyethylene units (usually a+c = 100 to 200 ethylene oxide and b = 15 to 50 propylene oxide units).

'Synperonic' PE/F127

Block copolymer of ethylene oxide and propylene oxide with 500 ppm w/w BHT added as antioxidant.

GENERAL CHARACTERISTICS	: *Classification*	: *Nonionic surfactant*
	Density at 77 Deg C, g/ml	: *1.05*
	Viscosity at 77 Deg C, mPa.S	: *3120*
	Flash point, open cup, Deg C	: *255*
SOLUBILITIES	: *Soluble in water, ethanol and toluene*	
	Insoluble in kerosene and ethylene glycol	
SPECIFICATIONS	: *Appearance at 20 Deg C*	: *White flakes*
	Colour of 25% aqueous solution, Hazen	: *Max 20*
	Water, % w/w	: *Max 0.75*
	Melting point, Deg C	: *Min 52*
	Hydroxyl number, mgKOH/g	: *11.5 - 8.5*
	pH, 2.5% aqueous solution	: *5.0 - 7.5*
	Arsenic, ppm w/w	: *Max 1*
	Heavy metals, ppm w/w	: *Max 15*
	Sulphated Ash. % w/w	: *Max 0.2*

The word 'Snyperonic' is a trade mark, the property of Imperial Chemical

Industries plc.

Formulation Experiment

8 mg DHEA was placed into 4 vials to each of which 2ml saline was added (4mg DHEA per ml). This was followed by the addition to each tube of 0.1, 0.05, 0.025 and 0 ml of polyxamer 407 (2mg/ml) corresponding to 0.2, 0.1, 0.05 and 0 mg poloxamer. All samples were stirred magnetically at room temperature for 24 h.. They were subsequently bath sonicated for 3 h. and allowed to stand. It was immediately obvious that only the sample with 0.2 mg of poloxamer 407 had the milky appearance and clarity under light. This sample was examined 3 days later and following its resuspension after vigorous mixing was injected I.V. into mice. There was no obvious adverse effect.

Experiment

DHEA (80 mg) was suspended in 5 ml 0.9% NaCl (saline) (DHEA aggregates were visible at this stage). Samples of 1.0, 0.75, 0.5 and 0.25 ml of the suspension (corresponding to 16, 12, 8 and 4 mg micronized DHEA) were made up to 1.0 ml with saline and mixed with 0.2 ml (0.2 mg) of a poloxamer 407 solution (1 mg/ml saline). Final concentrations of DHEA were

aprox. 8, 6, 4 and 2 mg/ml.

All samples were probe-sonicated (0.75 inch tip) for a total of 4-5 min (30 sec sonication periods with 30 sec. rest intervals) at room temperature. The sonicated samples were milky in appearance but were clear when a thin layer was observed under light. Samples tended to precipitate with time but it was possible to bring them into a milky (clear) state by vigorous mixing on a whirl mixer for 10-20 sec..

Mice (25 g each) injected I.V. with 0.2 ml of each suspension appeared to tolerate the injected material. 10 days later the same mice were re-injected with the same suspensions (which had precipitated but were cleared by mixing as above before injection). Again no adverse effects were observed. These in vivo tests were carried out in order to establish that particle size was too small for significant blockage of lung capillaries to occur in mice.

Two patients were administered the material as detailed in the following protocol with the micronized colloidal suspension of DHEA formulated as outlined in experiments above.

A CLINICAL TRIAL OF THE SAFETY AND EFFECTIVENESS OF INTRAVENOUSLY ADMINISTERED DEHYDROEPIANDROSTERONE IN PATIENTS WITH HIV INFECTION

PROTOCOL SUMMARY

TITLE: A Clinical Trial of Intravenously Administered DHEA Specially Formulated for Persons with HIV Infection.

INDICATION: Treatment of HIV-1 infection.

TYPE OF STUDY: Phase I/II Clinical Trial.

STUDY OBJECTIVES:

a. Determine the safety and tolerance of IV administered DHEA in persons with advanced HIV disease.

b. Determine the effect of IV administration of DHEA on measures of HIV Viral Load including quantitative viral dilution. HIV micro-culture of PBMC and also effects on the serum PCR (RNA) levels together with HIV p24 antigen (by acid dissociation method).

c. Determine the immune and toxicological effects of IV administered DHEA.

d. Determine the pharmokinetics of IV administered DHEA.

INCLUSION CRITERIA

a. Age 18 years or older;

b. HIV-1 seropositive;

c. A CD4+ -T-lymphocyte count of 50 to 300 cells/mm^3 within one month prior to study entry, measured on

two separate occasions 72 hours to 28 days apart;

d. *The following baseline laboratory values:*
   Hemoglobin >9g/dl
   WBCs > 1500 cells/ul
   Neutrophils > 1000 cells/ul
   Platelets 25,000 cells/ul
   Bilirubin < 2.0mg/dl
   AST, ALT, Alkaline phosphatase < 5 x upper limit of normal
   Creatinine < 1.5mg/dl;

e. *A history of prior anti-retroviral therapy as follows:*

   i. *No prior therapy. These patients may not begin other retroviral therapies during the first six weeks of this study. This period will allow for the effectiveness of DHEA at lowering the HIV viral load levels to be determined by the Principal Investigator. If Viral Load levels are demonstrated to be declining it would be envisaged that patients would refrain from other retroviral therapies for the complete duration of the trial (28 weeks).*

   ii. *In patients with a prior history of anti-retroviral therapy using AZT, ddI, ddC or d4T who are not receiving such therapy at study entry, these patients must have discontinued this medication more than 30 days prior to study entry. These patients may not begin anti-retroviral therapy during the first 6 weeks of this trial and should follow the direction of the Principal Investigator as per (i) above;*

g. *Use of suitable contraception by women of childbearing potential (requires one negative serum pregnancy test, beta-HCG, within one week prior to study entry in women of childbearing potential).*

EXCLUSION CRITERIA:

a. Previous treatment with chemotherapeutic agents within eight weeks of enrolment;

b. Active, major infection, including AIDS-defining opportunistic infection, or other life-threatening medical crisis;

c. Pregnant or breast-feeding;

d. Any condition which, in the investigator's opinion places the patient at undue risk or jeopardises the objectives of the trial;

e. Receiving immunomodulatory therapies including interferon or pharmacological doses of steroids at entry into the study;

SAFETY MEASURES: Biweekly analysis up to week 4 of the study of the following parameters:-

i. Documentation and assessment of adverse events

ii. Hematology

iii. Clinical chemistries and urinalysis

iv. Assessment of the immune responses resultant from DHEA.

EFFECTIVENESS MEASURES:

Reduction in viral load will be established by HIV titre (and time to positive culture) in quantitative, dilutional PBMC microcultures.

Additional measures of viral load will include HIV-p24 antigenemia, and HIV-RNA PCR (cell free, serum).

Improvements in immune response will be measured as changes from baseline in CD4/CD8 ratio. Clinical lymphocyte counts, percent WBC, increases in Interleukin 2 levels together with documenting the possible decreases in Interleukin 10 and 6 which would demonstrate the ability of DHEA to cause the patients immune system to move to a T_H-1 status.

Clinical benefit will be assessed by change in total body weight, Karnofsky performance score, and amelioration of signs and symptoms of disease present at baseline.

The remission or incidence of new opportunistic infection will be summarised.

STUDY DESIGN:

Open-label, daily intravenous administration of a single dose per patient, with review and assessment of the dosage schedules and efficacy after therapy for 4 weeks, dosage levels initially 100mg/day and 200mg/day, with follow-up laboratory analysis at weeks 0, 2, 4, 12 and 24 weeks. 9

STUDY SIZE:	*20 Patients (total)*	*10 Patients @ 100mg/day for 28 days therapy.* *10 Patients @ 200mg/day for 28 days therapy.*

TEST ARTICLES:

Test Drug:	*Dehydroepiandrosterone particle size distribution, 87%: < 5um, 100%: < 15 um, sterile filter and prepared for IV. administration by suspension in a colloidal formate using block polymer "Lutrol".*
Control Drug:	*None.*
Placebo:	*None.*

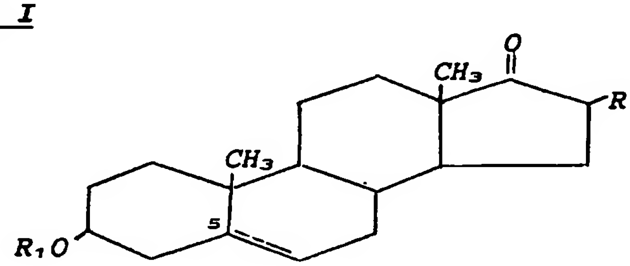
Patient data required before and after administration of DHEA

	Screen -4 Wks	Weeks					
		0	2	4	12	18	24
Physical Exam & Medical History	X	X	X	X	X		X
Urinalysis	X	X	X	X	X		X
Glucose	X	X	X	X	X		X
Neopterin	X	X	X	X	X		X
Beta2-microglobulin	X	X	X	X	X		X
RBC	X	X	X	X	X		X
hb	X	X	X	X	X		X
WBC	X	X	X	X	X	X	X
Platelet	X	X	X	X	X	X	X
T Cell Panel	X	X	X	X	X	X	X
HIV Viral Load PBMC Culture	XX	XX	XX	XX	XX		XX
p24 Antigen	X	X	X	X	X		X
Creatinine	X	X	X	X	X		X
SGOT	X	X	X	X	X	X	X
SGPT	X	X	X	X	X	X	X
IgG	X	X	X	X	X		X
IgA	X	X	X	X	X		X
IgM	X	X	X	X	X		X
Cutaneous DTH Responses	X	X	X	X	X		X
Hairy Leukoplakia & Aphthous Ulcers (Report, measurement & photograph)	X	X		X	X		X
DHEA	X	X	X	X	X		X
DHEAS	X	X	X	X	X		X
Testosterone	X	X	X	X	X		X
17 Ketosteroids	X	X	X	X	X		X
Interleukin 10	X	X	X	X	X		X
Interleukin 2	X	X	X	X	X	X	X
Interleukin 6	X	X	X	X	X		X
PCR (RNA) (Cell free, serum)	X	X	X	X	X		X
γ Interferon	X	X	X	X	X	X	X

I CLAIM

1. A dosage form for therapeutic delivery of a pharmaceutical formulation of compounds having the general formula (I) (as identified herein) in combination with a block polymer. This novel formulation containing a block polymer produces a colloidal suspension of the compound(s) of FORMULA I.

FORMULA I



(I)

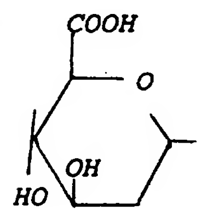
in which R is a hydrogen or bromine atom, and R_1 is a hydrogen atom, an SO_2OM group wherein M is a hydrogen or sodium atom, a sulphatide group

$$-SO_2O-CH_2.\underset{\substack{|\\ O.CO.R_2}}{CH}.CH_2O.CH.R_3$$

a phosphatide group

$$-\overset{\substack{O\\ \|}}{\underset{\substack{\|\\ O}}{P}}-O.CH_2.\underset{\substack{|\\ O.CO.R_2}}{CH}.CH_2.O.CO.R_3$$

wherein each of R_2 and R_3, which may be the same or different, is a straight or branched chain alkyl radical of 1 to 14 carbon atoms, or a glucuronide group



wherein the broken line represents an optional double bond, and the hydrogen atom at position 5 is present in the α- or β- configuration or the compound comprises a mixture of both configurations.

When R_1 is other than a hydrogen atom, the compounds are conjugated compounds.

Preferably in the compound of formula (I), R and R_1 are each hydrogen. An especially preferred compound is dehydroepiandrosterone wherein R and R_1 are each hydrogen and the double bond in present.

Additionally the compound of Formula I is 16α-bromoepiandrosterone, wherein R is Br, R_1 is H and the double bond is present. Another preferred compound of

Formula I is etiocholanolone wherein R and R_1 are each hydrogen and the double bond is absent.

Other preferred compounds of Formula I are dehydroepiandrosterone sulphate, wherein R is H, R_1 is SO_2-OM and M is as hereinbefore defined and the double bond is present, and 5ß-androstan-3a-ol-17-one.

Alternatively, Formula I is inclusive of compounds selected from dehydroepiandrosterone sulphatides, phosphatides or glucuronide wherein R is H, and R_1 is a sulphatide, phosphatide or glucuronide group as hereinabove defined, and the double bond is present. In particular when R_1 is not hydrogen the compounds are DHEA conjugates such as hexyl sulfate, dodecyl sulfate, octadecyl sulfate, octadecanolyglycol sulfate, O-dihexadecyclglycaro sulfate, hexadecane sulfonate, dioctadecanoylglycero phosphate, O-hlhexadecylglycero phosphate.

2. A dosage form according to claim 1 wherein the therapeutic administration is by intravenous delivery to humans or animals.

3. A dosage form according to claim 1 wherein the

therapeutic administration is by eye drop delivery.

4. A dosage form according to claim 1 wherein the therapeutic administration is by transdermal delivery.

5. A dosage form according to claim 1 wherein the therapeutic administration is by nasal or oral administration.

6. A dosage form according to claim 1 wherein the therapeutic administration is by suppository delivery.

7. A pharmaceutical formulation according to claims 1 to 6 wherein the block polymer is a polycondensates of ethylene oxide and propylene oxide, of general formula:

$$HOCH_2\text{-}CH_2(CH_2\text{-}CH_2O)_a\text{-}(CH(CH_3)\text{-}CH_2O)_b\text{-}(CH_2\text{-}CH_2O)_c\text{-}H$$

8. A pharmaceutical formulation according to claim 7 wherein the block polymer is known as "Synperonic" PE/F127 or Poloxamer 407 or Lutrol F127.
(Synperonic is a trade mark of Imperial Chemicals Industries - Lutrol is a trade mark of BASF)

9. A dosage form according to claim 1 where in addition to the 17-ketosteroid and the block polymer there is contained Butylated hydroxytoluene {2,6-di-tert-butyl-4-methylphenol} (BHT) added as antioxidant.

10. A dosage form according to claims 1 to 9 which contains metoclopramide (Monohydrochloride).

11. A dosage form according to claims 1 to 10 for the treatment of viral infections.

12. A dosage form according to claim 1 to 10 for the treatment of immune suppression.

13. A dosage form for therapeutic delivery of a cytotoxic compound of a steroid chemical nature in combination with a block polymer.

14. A dosage form according to claim 13 wherein the cytotoxic compound is an esterified oestrogen.

15. A dosage form according to claims 13 and 14 for neoplastic therapy. Wherein the compounded formulation reduced residence time in the circulatory system thus reducing side effects to organs not requiring direct treatment.

IE PATENT APPLICATION No. 930811

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Patents Office

ABSTRACT

The present invention relates to the use of aliphatic carbocyclic compounds that have pendant hydroxy groups for incorporating water-insoluble molecules as hydrophilic components of liposomal drug delivery systems for human or animal treatment.

In particular this patent describes a liposomal drug delivery system wherein the hydrophilic component contains in part a 17-ketosteroids along with aliphatic carbocyclic compounds that have pendant hydroxy groups, such as cyclodextrin (CD), and the likes, as well as mixtures thereof. In particular the 17-ketosteroids is Dehydroepiandrosterone(DHEA) and the cyclodextrin is hydroxypropyl-beta-cyclodextrin. The cyclodextrin is used with the 17-ketosteroid to give it hydrophilic characteristics.

The present invention also relates to the use of certain 17-ketosteroids as hydrophobic components of liposomal drug delivery systems. As hydrophobic elements, of liposomes, the 17-ketosteroids have been discovered to have beneficial rapid drug delivery characteristics hitherto unknown.

930811

APPLICATION No.........................

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PATENT APPLICATION

INVENTOR: PATRICK T. PRENDERGAST

TITLE: LIPOSOMAL FOR CONTAINING ALIPHATIC CARBOCYCLIC COMPOUNDS WITH PENDANT HYDROXY GROUPS

Patrick T. Prendergast

BACKGROUND TO THE INVENTION

Any prescribed drug dose is the result of compromise. On the one hand, all drugs are potentially poisonous, suggesting that the least possible amount should be administered. On the other hand, drugs become diluted in the blood and large amounts are degraded, taken up by healthy tissues or excreted without ever reaching the site of disease. Such wastage increases the need for high doses. Physicians balance these opposing pressures by prescribing doses they think will be high enough to control the patient's problem but low enough to avoid causing unacceptable damage to healthy tissues.

To reduce the risk and inefficiency associated with such guesswork, many laboratories are now developing drug-delivery systems that alter the pathways by which drugs travel through the body. The goal is to deliver the needed dose of medicine to diseased tissues but to bypass healthy ones, thereby improving the drug's ratio of effectiveness to toxicity. One highly promising approach to achieving this goal is the loading of medication into liposomes, which are microscopic sacs made of the very phospholipids that constitute cell membranes.

Liposomes can be filled with a variety of medications and, because of their similarity to cell membranes, are not toxic. They also protect their loads from being diluted or degraded in the blood. As a result, when the liposomes reach diseased tissues, they deliver concentrated doses of medication. Liposomes containing a variety of drugs have been shown in many animal studies, and in some clinical tests, to be more effective and less toxic than free drugs.

Alec D. Bangham of the Agricultural Research Council's Institute of Animal Physiology in Cambridge, England, inadvertently produced the first liposomes in 1961 while evaluating the effect of phospholipids on blood clotting. When Bangham put water in a flask containing a phospholipid film, the water forced the molecules to arrange themselves into what he later discovered were microscopic closed vesicles composed of the bilayered (two-molecule-thick) phospholipid membrane surrounding water entrapped from the environment.

Phospholipids form closed, fluid-filled spheres when they are mixed with water in part because the phospholipid molecules are amphipathic: they have a hydrophobic (water-insoluble) tail and a hydrophilic (water soluble), or "polar", head. Two fatty acid

chains, each containing from 10 to 24 carbon atoms make up the hydrophobic tail of most naturally occurring phospholipid molecules. Phosphoric acid bound to any of several water-soluble molecules composes the hydrophilic head. When a high enough concentration of phospholipids is mixed with water, the hydrophobic tails spontaneously herd together to exclude water, whereas the hydrophilic heads bind to water.

The resultant bilayer in which the fatty acid tails point into the membrane's interior and the polar head groups point outward is called a liposome. The polar groups at one surface of the membrane point towards the liposome's interior and those at the other surface point toward the external environment. It is this remarkable reactivity of phospholipids to water that enables workers to load medications into liposomes. As a liposome forms, any water-soluble molecules that have been added to the water are incorporated into the aqueous spaces in the interior of the spheres, whereas any lipid-soluble molecules added to the solvent during vesicle formation are incorporated into the lipid bilayer.

Liposomes employed for drug delivery typically range in diameter from 250 angstrom units to

several micrometers (the diameter of a red blood cells is roughly 10 micrometers) and are usually suspended in a solution. They have two standard forms: "onion-skinned" multilamellar vesicles (MLV's), made up of several lipid bilayers separated by fluid, and unilamellar vesicles, consisting of a single bilayer surrounding an entirely fluid core. The unilamellar vesicles are typically characterised as being small (SUV's) or large (LUV's).

Under appropriate circumstances liposomes can be absorbed into almost any cell type. Once they have been adsorbed the spheres may be endocytosed, or swallowed up, by some cells. Adsorbed liposomes can also exchange lipids with cell membranes and may at times be able to fuse with cells. When fusion takes place, the liposomal membrane is integrated into the cell membrane and the aqueous contents in the cell.

SUMMARY OF THE INVENTION

The present invention relates to the use of aliphatic carbocyclic compounds that have pendant hydroxy groups for incorporating water-insoluble molecules as hydrophilic components of liposomal drug delivery systems for human or animal treatment.

In particular this patent describes a liposomal drug delivery system wherein the hydrophilic component contains in part a 17-ketosteroid along with or combined with an aliphatic carbocyclic compounds that have pendant hydroxy groups, such as cyclodextrin (CD),and the likes, as well as mixtures thereof. In particular the 17-ketrosteroid is Dehydroepiandrosterone(DHEA) and the cyclodextrin is hydroxypropyl-beta-cyclodextrin. The cyclodextrin is used with the 17-ketosteroid to give hydrophilic characteristics. Additional therapeutic efficiency is obtained where DHEA is required as the active agent resulting from the fact that when DHEA is complexed with beta-cyclodextrin the compound is not readily transformed to DHEA-sulphate within the body.

The present invention also relates to the use of certain 17-ketosteroids as hydrophobic components of liposomal drug delivery systems. As hydrophobic

elements, of liposomes, the 17-ketosteroids have been discovered to have beneficial rapid drug delivery characteristics hitherto unknown. Liposomes having 17-ketosteroids as hydrophobic components are thus claimed to be ideal carriers for the delivery of any therapeutic agent and in particular toxic therapeutic agents wherein the therapeutic agent forms part or the total hydrophilic component of the liposome.

The preparation of suitable hydroxypropyl-beta cyclodextrins is described, inter alia, in International Journal of Pharmaceutics 29:73-82 (1986) and in Journal of Pharmaceutical Sciences 75 (6):571-572 (1986). Also known, and contemplated for the purposes of the present invention are the hydroxypropyl-beta-cyclodextrins that are polyesters of cyclodextrins and are obtained by condensation of an excess of hydroxypropylene oxide with beta-cyclodextrin as described in U.S. Pat. No. 3,459,731 to Gzamera et al. Historically, cyclodextrins (CDs) have been used extensively in the pharmaceutical industry in oral formulations, parenteral formulations and suppositories. In practical terms, CD complexes improve drug stability, enhance solubility, promote faster absorption, reduce local irritation and result in improved bio-availability.

This method of encapsulating hydrophobic molecules in the hydrophilic compartment of liposomes using cyclodextrins has definite advantages over the classical method of dissolving in organic solvents. Complexation is favoured in cold, concentrated CD solutions. Equilibrium is shifted in warm, dilute solutions and molecules of interest or guest molecules are released. Stability is conferred to the guest molecule by protecting it from degradation due to heat, sublimation, enzymatic sulphation, oxidation and/or light. Improved bio-availability of components that have been complexed also occurs because their homogeneous distribution is increased and their transfer into a molecular-dispersed state is facilitated upon release from the liposome. Many of the 17-ketosteroids function as hormones and include sex hormones or precursors thereof and hormones which control metabolism. Dehydroepiandrosterone (DHEA) is one such 17-ketosteroid which is a precursor of both androgens and estrogens and additionally has important metabolic effects. DHEA has been found to suppress some of the metabolic disorders and liver cirrhosis, and reduces pain in ischemic heart disease, especially in angina pectoris, by restricting tissue respiration. DHEA has been used in the treatment of menopause,

emotional instability, depression and stress. DHEA and related compounds are capable of reducing the colony forming ability of human peripheral blood mononuclear (PBM) cells infected with Epstein-Barr virus (a herpes virus) at concentrations of 10-100 uM (Carcinogenesis, Vol. 2, pp 883-886, 1981).

DHEA also inhibits complement activation and is therefore of value in the prophylaxis of Hereditary Angioneurotic Oedema (Hidvegi et al., Complement 1; 201, 1984). DHEA also prevents autoantibody formation in the murine model of Systemic Lupis Erythematosus (SLE) and many of the features of full-blown AIDS are considered to be similar to those of SLE (lucas et al., J. Clin. Invest., 75: 2091, 1985).

Recent studies in animals demonstrate that DHEA has beneficial effects in obesity and breast cancer. Schwartz Cancer Res. 39:1129 (1979); Schwartz Nutrition and Cancer, 3:46 (1981), DHEA also has been shown to have antihypercholesterolic effects in lowering lipid levels in rats. Ben-David et al., Proc. Soc. Exp. Biol. Med., 20 125:1136 (1967).

The importance of hypercholesterolemia, an elevated low-density lipoprotein (LDL) cholesterol level, as a major risk factor for the development of ischemic heart disease is widely accepted.

Barrett-Connors et al., New Engl. J. Med. 315:1519 (1986) showed that individuals with low circulating levels of DHEA-S die of heart disease at a higher rate than normal subjects. The oral administration of DHEA (1600 mg/day) reduces total serum cholesterol and LDL level by about 7.1 and 7.5 percent, respectively, in normal subjects.

The use of DHEA and other 17-ketosteroids as medication for the prophylaxis and therapy of a retrovirus infection or for complications arising therefrom, e.g., Acquired immune deficiency syndrome (AIDS) has been reported in SCRIP No. 1422, June 21, 1989, page 21 and in British Pat. Publication No, 2,204,237 by Colthurst, Ltd. Oral administrations of relatively large doses of 1 to 2 grams per day has been tested in AIDS patients and shown to improve their immune systems and lower viral HIV load. In such tests, DHEA was administered orally alone or in combination with immunomodulators. Liposomes carrying DHEA as a hydrophilic CD complex target HIV infected macrophages and Kuffer cells more directly and thus reduce the dosage required, this formulation prevent sulphation to inactivate DHEAS form in the plasma and deliver the required dosage to the infected target tissues.

Studies with Liposomal Dehydroepiandrosterone (DHEA) in Mice

Study 1: Fate of liposomal DHEA after i.v. injection into mice.

Liposomes composed of soy lecithin and incorporating DHEA (mixed with radiolabelled DHEA) were injected i.v. (0.25 ml containing 0.9 mg DHEA) into Balb/c inbred mice. Animals were bled from the tail vein at time intervals and killed at 24 hrs after injection. Blood plasma samples and tissues obtained at death were analysed for radioactivity (^3H). Results are shown in Tables 1 and 2.

Table 1: Clearance of liposomal DHEA from the circulation

Mouse	% of injected liposomal DHEA in total blood				
	2 min	30 min	2.5 hours	7 hours	24 hours
1	9.2	3.1	1.4	0.5	0.2
2	12.5	3.0	0.9	0.5	0.3
3	8.0	4.0	2.1	0.4	0.3
4	13.8	5.1	1.8	0.3	0.1

Comment: Most of the injected liposomal DHEA is removed from the circulation within the first 2 minutes.

Table 2: Tissue levels of liposomal DHEA 24 hours after i.v. injection.

Mouse	% of injected liposomal DHEA in total tissue			
	Liver	Spleen	Kidney	Lungs
1	0.6	< 0.1	0.1	< 0.1
2	0.4	< 0.1	0.2	< 0.1
3	0.3	< 0.1	0.1	< 0.1
4	0.5	< 0.1	0.0	< 0.1

Comment: Very little liposomal DHEA recovered in tissues at 24 hours.

Study 2: Blood and tissue levels of liposomal DHEA soon after i.v. injection.

This study was undertaken to (a) confirm the rapid clearance of liposomal DHEA observed in Table 1 and (b) measure tissue levels soon after injection. Results are shown in Table 3.

Table 3: Blood and tissue levels of liposomal DHEA min after i.v. injection

Mouse	% of injected liposomal DHEA in total blood or tissue				
	Blood	Liver	Spleen	Kidney	Lungs
1	5.5	18.5	1.0	8.0	2.1
2	8.5	14.7	2.1	6.5	2.5
3	6.6	21.6	1.8	4.8	1.8
4	4.5	20.7	1.5	8.2	1.5

Comment: Blood contains again very little of the injected radioactivity 5 min after injection. Tissues contain more at 5 min than they did at 24 hrs. (see Table 2).

Study 3: Mouse survival after 5 intravenous injections of liposomal DHEA.

Five mice were injected i.v. once every day for 5 days with 0.9 mg liposomal DHEA. 24 hrs. after the last injection mice appeared healthy and remained so for at least two weeks.

Intravenous injection of liposomal DHEA (0.9 mg per mouse) led to the rapid removal of the drug from the circulation (nearly 90% removed within the first 2 min). This is in contrast with previous findings with liposomes of similar size (diameter less than 250 nm) where over 50% of the dose is recovered in blood circulation 2 min after injection. Such rapid clearance of DHEA liposomes suggests the possibility of the receptor for the drug, the latter probably being available on the liposomal surface. Examination of tissues revealed that the liver took up more of liposomal DHEA than spleen, kidneys and lungs. This is consistent with previous observations of liposomal fate. With regard to possible liposomal DHEA toxicity, this was not apparent in mice injected daily over five days.

The steroid dehydroepiandrosterone (DHEA) is solublised in a 45% solution of 2-hydroxy-propyl-beta-cyclodextrin (HPBCD) to a concentration of 47.8mg DHEA/ml of solution in water. This solution is then formulated into liposomes.

I CLAIM:

1. A liposomal drug delivery system wherein the hydrophobic component of the liposome is composed, in whole or in part,of a 17-ketosteroid.

2. A drug delivery system according to claim 1 wherein the 17-ketosteroid is dehydroepiandrosterone(DHEA).

3. A liposomal drug delivery system wherein a 17-ketosteroid is used to target the liposome to specific body tissue.

4. A drug delivery system according to claim 3 wherein the 17-ketosteroid is dehydroepiandrosterone (DHEA).

5. A drug delivery system according to claim 1,2,3,4 wherein the therapeutic agent is removed rapidly form circulation.

6. A liposomal drug delivery system wherein the hydrophilic component contains in whole or in part a 17-ketosteroids alond with one or more aliphatic carbocyclic compounds that have pendant hydroxy groups.

7. A drug delivery system according to claim 6 wherein the aliphatic carbocylic compounds that have pendant hydroxy groups is hydroxypropyl-beta-cyclodextrin (HPBCD).

8. A drug delivery system according to claim 6 wherein the 17-ketosteroid is dehydroepiandrosterone(DHEA).

9. A liposomal drug delivery system wherein the therapeutic agent is totally or partially a 17-ketosteroids.

10. A drug delivery system according to claim 9 wherein the 17-ketosteroid is dehydroepiandrosterone(DHEA).

11. A drug delivery system according to claim 10 wherein the therapeutic agent is complexed with one or more aliphatic carbocyclic compounds that have pendant hydroxy groups thus restricting the enzymatic transformation of DHEA to DHEA-sulphate within the body.

12. A liposomal drug delivery system wherein the hydrophilic and hydrophobic components contains in whole or in part a 17-ketosteroids along with one or more aliphatic carbocyclic compounds that have pendant hydroxy groups.

13. A drug delivery system according to claim 12 wherein the aliphatic carbocyclic compounds that have pendant hydroxy groups is hydroxypropyl-beta-cyclodextrin (HPBCD).

14. A drug delivery system according to claim 12 wherein the 17-ketosteroid is dehydroepiandrosterone(DHEA).

15. A liposomal drug delivery system wherein the hydrophilic component contains in whole or in part a complex of a therapeutic agent with one or more aliphatic carbocyclic compounds that have pendant hydroxy groups.

16. A drug delivery system according to claim 15 wherein the therapeutic agent is interleukin-2.

17. A drug delivery system according to claim 15 wherein the therapeutic agent is tumor necrosis factor.

18. A drug delivery system according to claim 15 wherein the therapeutic agent is insulin.

19. A drug delivery system according to claim 15 wherein the therapeutic agent is Alpha-fetoprotein or antibodies to parts thereof.

20. A drug delivery system according to claim 15 wherein the aliphatic carbocyclic compounds that have pendant hydroxy groups is hydroxypropyl-beta-cyclodextrin (HPBCD).

21. A drug delivery system according to claim 15 wherein the alphatic carbocyclic compounds that have pendant hydroxy groups is alpha-cyclodextrin.

22. A drug delivery system according to claim 15 wherein the aliphatic carbocyclic compounds that have pendant hydroxy groups is beta-cyclodextrin.

23. A drug delivery system according to claim 15 wherein the aliphatic carbocyclic compounds that have pendant hydroxy groups is gamma-cyclodextrin.

ABSTRACT

The present invention relates to the use of aliphatic carbocyclic compounds that have pendant hydroxy groups for incorporating water-insoluble molecules as hydrophilic components of liposomal drug delivery systems for human or animal treatment.

In particular this patent describes a liposomal drug delivery system wherein the hydrophilic component contains in part a 17-ketosteroids along with aliphatic carbocyclic compounds that have pendant hydroxy groups, such as cyclodextrin (CD), and the likes, as well as mixtures thereof. In particular the 17-ketosteroids is Dehydroepiandrosterone(DHEA) and the cyclodextrin is hydroxypropyl-beta-cyclodextrin. The cyclodextrin is used with the 17-ketosteroid to give it hydrophilic characteristics.

The present invention also relates to the use of certain 17-ketosteroids as hydrophobic components of liposomal drug delivery systems. As hydrophobic elements, of liposomes, the 17-ketosteroids have been discovered to have beneficial rapid drug delivery characteristics hitherto unknown.